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FURTHER OBSERVATIONS ON THE COMPLEMENT FIXATION TEST IN THE DIAGNOSIS OF LUES IN THE MILITARY SERVICE: AN ANALYSIS OF 3,950 TESTS.*†

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In the *Journal of Experimental Medicine* (1910, 12, p. 726), I published the results obtained from the use of the Wassermann complement fixation test in the diagnosis of lues in the army, giving the data of the cases examined to September 10, 1910, the method having been adopted in this laboratory November 1, 1909. In this paper I desire to give the results we have obtained with this test from November 1, 1909, to June 30, 1911, believing that the large number of cases discussed proves conclusively the great value of the test in the diagnosis of lues both in the military service and civil life.

Technic.—The technic employed for the complement fixation test in this laboratory is practically that recommended by Noguchi, the only departure from his technic being the use of an alcoholic extract of fetal luetic liver as antigen, and the use of inactivated

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sera. It is impossible for us to use active serum as we make tests for army posts situated at some distance from the laboratory, in consequence of which most of the serum is from two to three or four days old before it can be tested. For this reason we have preferred to use an alcoholic extract of luetic liver as antigen instead of the acetone-insoluble fraction recommended by Noguchi, and in order to avoid the thermolabile anticomplementary bodies and the non-specific reactions sometimes observed when an alcoholic extract is used as antigen, the sera are always inactivated just before making the test. I have compared this method in a large number of cases with the acetone-insoluble antigen of Noguchi, using inactivated sera, and have found that the results are identical except in cases giving a weak plus-minus reaction, when the acetone-insoluble fraction gave a slightly larger percentage of positive results. However, as such reactions are of little value in diagnosis we have seen no reason to abandon the use of the alcoholic extract when inactivated serum is used. If it were possible for us to use active serum, the acetone-insoluble fraction would give slightly better results than the alcoholic extracts, as has been shown by numerous investigators.

Before adopting the complement fixation test as a method of diagnosis of lues in the army, careful experiments were conducted in regard to the keeping qualities of human sera, as some authorities had reported that certain specimens of normal serum, if kept for several days, would give a positive reaction.

In all, 50 normal sera were tested, the blood being collected in Wright tubes and the serum allowed to separate, after which it was pipetted into suitable glass containers and sealed. Enough of these tubes were prepared to enable us to make a complement fixation test upon each serum at intervals of one week, for a month. Of the 50 sera examined, all gave a negative reaction at the time of collection and 43 were still negative at the end of a month, while seven had developed anticomplementary bodies which produced complete inhibition of hemolysis in both antigen and control tubes. As an alcoholic extract was used as antigen the sera were always inactivated before the test was made, but in two sera tested before inactivation, both being about two days old, a positive reaction was obtained. Both of these sera gave a negative reaction after inactivation, and neither was contaminated by bacteria. This observation confirms that of other investigators who have found that non-specific reactions may occur in testing active sera when an alcoholic extract is used as antigen.

The experiments have convinced us that as long as normal sera remain free from bacterial contamination, and are inactivated before use, they do not give a positive reaction, even though kept at room temperature for a month. However, in some of the sera, both thermolabile and thermostabile anticomplementary bodies developed which caused inhibition of hemolysis in both antigen and control tubes. The experiments proved that a positive reaction cannot occur in normal sera, if proper precautions are taken, for a period of time far in excess of any delay that may occur before the specimens reach the laboratory.

As stated, the antigen employed has been an extract of luetic liver in absolute alcohol. The crude extract, prepared according to Noguchi, is diluted just before use with nine parts of normal salt solution, and of this 0.1 c.c. is used for each tube, provided this dose is found sufficient after titration of the diluted antigen. I have used a large number of antigens prepared from normal tissues but have not found any as satisfactory as those prepared from fetal luetic liver. It should be remembered that in using an alcoholic extract as antigen the serum to be tested *must* be inactivated.

The blood suspension used is a one per cent suspension of human red blood corpuscles prepared as recommended by Noguchi.

The amboceptor is prepared by immunizing rabbits to human red blood corpuscles in the manner recommended by Noguchi. When the serum has been found by titration to be of sufficient strength the animals are bled and filter paper is impregnated with their serum. Thus preserved the serum keeps very much longer than when it is used in the fluid condition, and I have found that amboceptor paper loses but little strength even for periods as long as six months. The paper should be titrated at least every two weeks in order to be sure of its exact strength.

The complement used has been guinea-pig serum not over 24 hours old. Serum kept from 12 to 20 hours is stronger in complement than fresh serum or serum over 20 hours old. I desire to emphasize strongly the necessity of titrating the complement before using it, for guinea-pig serum varies considerably in complementary qualities. It is also necessary to use the serum of at least two pigs, as occasionally the serum of one pig is so weak in complement that it cannot be used for the test.

The method of making the test will not be discussed as it is fully described in the work of Noguchi upon the serum diagnosis of syphilis.

General results.—The tests made in this laboratory may be divided into the following three classes: *Group 1:* Cases in which the clinical diagnosis was lues or suspected lues, or in which there was a history of previous infection (this includes latent cases). *Group 2:* Cases in which clinical symptoms or diagnosis were of some other disease. *Group 3:* Normal individuals.

The total number of individuals examined to June 30, 1911, has been 2,435, while 1,515 repetitions of the test have been made as a guide to treatment, making a total of 3,950 tests. In addition to these, several hundred tests have been made in research work upon the subject of complement fixation. It is believed that the value and accuracy of the technic employed is amply demonstrated by the results obtained in this large series of cases, especially as these results were controlled by repetitions of the test in over 500 patients who were placed upon treatment after the primary test.

Of the 2,435 individuals tested, 1,322 gave a positive reaction, and 1,113 a negative reaction. In the following pages the conventional signs ++, +, and +− are used to indicate varying degrees of inhibition of hemolysis: thus, ++ indicates absolute inhibition, + indicates at least 50 per cent of inhibition, and +− any well marked degree of inhibition below 50 per cent. A diagnosis of lues should not be based upon a +− reaction unless there is a clear clinical history of infection or the patient is on specific treatment.

The 2,435 cases were divided as follows: Group 1, 1,661 cases; Group 2, 618 cases; and Group 3, 156.

GROUP 1: *Cases in which the diagnosis was lues or suspected lues, etc.*—Of this group of cases 1,661 were examined, of which 1,315, or

79.1 per cent, gave positive results. The cases were divided as regards the stage of the disease as shown in Table 1.

TABLE 1.

RESULTS OF THE COMPLEMENT FIXATION TEST IN 1,661 CASES OF LUES.

Stage of the Disease	No. of Cases	Positive	Negative	Percentage Positive
Primary	293	219	74	74.7
Secondary	723	658	65	91.
Tertiary	217	185	32	85.2
Latent	296	228	168	57.5
Congenital	19	17	2	89.4
Parasyphilitic	13	8	5	62.3
Totals	1,661	1,315	346	79.1

However, the above table is not a fair statement of the results actually obtained with the complement fixation test in our cases for there were 53 individuals tested in the primary stage of the disease at a period too early for the reaction to have appeared, while in 37 of the secondary cases giving a negative result vigorous specific treatment had been administered for weeks or months. Deducting these cases from the totals given in Table 1, we have 1,571 cases in Group 1, of which 1,315, or 83.7 per cent, gave a positive result. The cases were divided regarding the stage of the disease as shown in Table 2.

TABLE 2.

RESULTS OF THE COMPLEMENT FIXATION TEST AFTER DEDUCTING CASES MENTIONED IN TEXT.

Stage of Disease	No. of Cases	Positive	Negative	Percentage Positive
Primary	240	219	21	91.2
Secondary	686	658	28	95.9
Tertiary	217	185	32	85.2
Latent	396	228	168	57.5
Congenital	19	17	2	89.4
Parasyphilitic	13	8	5	62.3
Totals	1,571	1,315	256	83.7

Before considering the results in the various stages a little more in detail it is interesting to compare the percentage of positive results in these 1,571 cases of lues with the percentage of positive results obtained in the 361 cases of lues reported in the previous paper alluded to, as such comparison demonstrates the influence of numbers upon the percentage of positive results. In making this comparison the 13 parasyphilitic cases are deducted from the

1,571 cases, as this class was not included in the previous report. Table 3 gives the result in both series in the various stages of the disease.

TABLE 3.

COMPARISON OF THE RESULT OF THE COMPLEMENT FIXATION TEST IN SERIES 1 AND 2.

Series 1: 361 Cases			Series 2: 1,558 Cases		
Stage of Disease	No. Cases	Percentage Positive	Stage of Disease	No. Cases	Percentage Positive
Primary	76	85.5	Primary	240	91.9
Secondary	147	97.2	Secondary	686	95.9
Tertiary	74	82.	Tertiary	217	85.2
Latent	55	72.	Latent	396	57.5
Congenital	9	88.8	Congenital	19	89.4
Totals	361	87.7	Totals	1,558	83.8

It is evident from this table that the percentage of positive results in the primary, tertiary, and congenital cases increases with the number of individuals examined and that the percentage decreases slightly in the secondary stage and considerably in the latent stage. The decrease in the percentage of positive results in both these stages was due to the fact that a larger number of cases having had some specific treatment were tested, and such factors should always be considered in comparing the results of various observers with the complement fixation test.

Results in the primary stage.—All cases have been considered as primary which have presented the initial lesion and which were tested before the development of secondary symptoms. Of this class of cases 293 were examined, of which 219, or 74.7 per cent, were positive. These figures, however, include 53 individuals who were tested within two to four days after the appearance of the initial lesion, too early for the reaction to appear, and deducting these we have 240 cases, of which 219, or 91.2 per cent, were positive. The percentage of positive results in primary lues has varied from 38.6 per cent to 100 per cent, as given by various observers, and our results agree with those of the majority of observers who have used both the original Wassermann technic and the Noguchi modification. Some cases have never given a reaction in the primary stage, the reaction only appearing after the development of secondary symptoms.

Time of the appearance of the reaction.—In 140 of our cases we have been able to determine the date of the appearance of the positive reaction, and Table 4 gives the data in these cases. There have been a large number of cases which were tested within a month to six weeks after the appearance of the initial lesion, but the cases here recorded are the only ones in which we can be sure, within approximate limits, of the date of the appearance of a positive reaction. The intensity of the reaction is also indicated in the table.

TABLE 4.

SHOWING DATE OF APPEARANCE OF REACTION IN 140 CASES OF LUES.

Time after Initial Lesion	No. of Cases	Character of Reaction			Time after Initial Lesion	No. of Cases	Character of Reaction		
		++	+	+−			++	+	+−
5 days	6	2	3	1	17 days	3	3	..	..
6 days	1	..	..	1	18 days	2	2	..	..
7 days	4	3	..	1	19 days	2	2	..	..
8 days	4	2	2	..	3 weeks	34	24	4	6
10 days	3	1	1	1	1 month	25	18	5	2
11 days	7	4	3	..	5 weeks	7	2	5	..
13 days	4	3	1	..	6 weeks	16	9	5	2
2 weeks	8	6	1	1	7 weeks	2	1	1	..
16 days	2	1	1	..	2 months	10	6	4	..

An extended discussion of this table is not necessary as it is self-explanatory. It will be noticed that the date of the appearance of the first positive reaction varied all the way from five days to two months, the greater number of cases becoming positive in the third and fourth week after the appearance of the initial lesion. No less than six cases became positive in five days, while 10 did not become positive until two months had elapsed, and this well illustrates the great variation in the time of appearance of the reaction.

Results in the secondary stage.—There were 723 individuals examined during the secondary stage of lues, of whom 658, or 91 per cent, gave a positive result. From these should be deducted 37 individuals who had received vigorous specific treatment for weeks or months and who were receiving treatment at the time the test was made. Deducting these cases we tested 686 individuals in the secondary stage of lues, of whom 658, or 95.9 per cent, gave a positive result. It is evident that positive results are more frequent in the secondary than in the primary stage of lues, and our percentage of positive results agrees with that of the majority of observers. There have been a few cases presenting well marked secondary lesions in which a positive result was not obtained, although repeated tests were made, and I think it must be admitted that we occasionally encounter a patient presenting severe primary, secondary, or even tertiary lesions in whom the Wassermann reaction remains negative.

Results in the tertiary stage.—There were 217 individuals examined in this stage of the disease. Of these 185, or 85.2 per cent, gave a positive reaction. Many of the patients examined had had more or less specific treatment, but these cannot be eliminated as so few would be left that the figures would have little value. Great variations in the results obtained by different observers with the complement fixation test have been noted in this stage of the disease, but they have depended largely upon the class of cases examined, as some authorities have included cases presenting no tertiary symptoms in this class, while others have only considered those in which marked symptoms were present. In nearly all of our cases some symptom of the tertiary stage was present at the time of the examination.

Results in latent lues.—In this class of cases are included all those in which no active symptoms of lues were present at the time of the examination but in which a clear history of infection was obtained. Almost all of these cases had received more or less specific treatment and the majority were tested in order to determine whether the disease had been cured. Of this class, 296 individuals were examined, of whom 228, or 57.5 per cent, gave a positive result. In most of these cases the only evidence which existed of lues was general glandular enlargement and in many even this symptom was absent. Our results are slightly higher in this class of cases than those given by the majority of observers, but the difference is slight, and indicates that in latent lues the technic recommended by Noguchi gives better results than that used in the original Wassermann test.

Congenital lues.—Only 19 cases of congenital lues have been tested, of which 17, or 89.4 per cent, gave a positive result. Our results are in agreement with those of the majority of observers, using either the original Wassermann technic or the Noguchi modification.

Results in parasyphilitic conditions.—Ten patients suffering from tabes and three from paresis were tested, the total 13 giving eight positive results, or 62.3 per cent. All the paretics gave a positive result and five, or 50 per cent, of the patients suffering from tabes. Here again our results agree with those of observers who have used the original Wassermann technic.

GROUP 2: This group includes all cases in which the patient was suffering from some other disease than lues. Of this group 618 individuals were examined, of whom seven, or one per cent, were positive. Three of the positive results were obtained in patients suffering from tertian malarial fever, the blood being tested during the febrile stage, and in all the blood became negative after the subsidence of the fever. In the other cases, four in number, three were diagnosed as pulmonary tuberculosis, and one as "undetermined fever." The three cases diagnosed as tuberculosis gave strong reactions and recovered under specific treatment. In two of these a history of luetic infection was afterward obtained, while in the other such an infection could not be excluded. In the case diagnosed as "undetermined fever" the exact nature of the disease could not be learned, but it was probably malarial fever.

GROUP 3: The blood of 156 individuals in good health and in whom luetic infection could be excluded was tested and a negative result was obtained in every instance.

The specificity of the test.—As a result of our experience I believe that the complement fixation test is specific for lues if such conditions as leprosy, malarial fever, scarlet fever, and frambesia can be excluded. It is unnecessary to discuss in detail the results which have been obtained with the test in these diseases, but it is well known that a certain percentage of patients suffering from them give a positive reaction whether the original Wassermann technic be used in making the test or the Noguchi modification. A few other conditions have been observed in which positive results were obtained in isolated cases, as carcinoma, tuberculosis, and sepsis, but the cases reported are infinitesimal in number, and do not vitiate the practical value of this test in the diagnosis of lues, as a latent luetic infection could not be excluded. I believe that a large percentage of positive results in non-luetic cases is proof of imperfect technic and that such reports must be viewed with suspicion.

If the diseases in which the complement fixation test has occasionally been found positive can be excluded, a double-plus or plus reaction is sufficient to enable one to diagnose the presence of lues. I am convinced that, under such conditions, the test is absolutely specific whether symptoms of the disease are present or not, and whether there is, or is not, a history of infection. In those cases in which, after the appearance of a suspicious lesion, the negative reaction becomes positive, a diagnosis of lues can be made without hesitation. On the other hand, a diagnosis of lues should never be made upon a plus-minus reaction alone.

The value of a negative reaction is not as great as that of a positive one. A considerable proportion of cases of lues do not give a positive reaction, even though symptoms are present, and for this reason the disease cannot be excluded on the strength of a negative result. In the interpretation of a negative result the history of the case, the symptoms present, and the amount of previous specific treatment must all be carefully considered.

The test as a control of treatment.—In a very large number of our cases the test has been used as a control of treatment both with mercury and with arsenobenzol (Salvarsan). Our results demonstrate the very great value of the test as an index of the efficacy of treatment, especially of treatment with arsenobenzol. At the present writing tests are being made upon over 700 patients who have been treated with this drug, the blood being tested every month in the majority of the cases. We have been able to trace the gradual disappearance of the reaction in treated cases and in a certain proportion its gradual reappearance before a clinical relapse occurred. The data derived from the test has made possible the intelligent use of this drug and of mercury, in the treatment of both initial infections and relapses.

The test has also demonstrated that even prolonged treatment with mercurials and potassium iodide does not cause the reaction to disappear permanently, although in many cases treatment with mercury, if the drug is being taken at the time of the examination, will render the test negative or only weakly positive. In our experience mercury should always be omitted for at least three to four weeks before the blood is tested, as otherwise negative results will often be obtained in positive cases.

In order to show the persistence of positive reactions after mercurial treatment I have compiled the results in a number of our cases and they are given in Table 5.

TABLE 5.

ILLUSTRATING THE PERSISTENCE OF THE COMPLEMENT FIXATION TEST AFTER MERCURIAL TREATMENT.

Method of Mercurial Treatment	Length of Treatment	No. of Cases	Intensity of the Reaction		
			++	+	+−
Internal	1 month	2	..	2	..
“	2 months	3	1	2	..
“	3 “	4	2	2	..
“	5 “	4	1	3	..
“	6 “	7	4	1	2
“	7 “	3	2	1	..
“	8 “	3	3	..	..
“	9 “	10	7	1	2
“	10 “	2	1	1	..
“	11 “	3	3	..	..
“	1 year	19	9	5	5
“	14 months	2	2	..	..
“	15 “	2	1	1	..
“	16 “	4	3	..	1
“	17 “	1	1	..	..
“	18 “	7	1	4	2
“	19 “	2	2	..	..
“	20 “	1	..	1	..
“	2 years	15	8	5	2
“	2.5 “	3	..	2	1
“	3 “	5	1	2	2
“	4 “	1	1	..	..
Internal*	7 “	1	..	1	..
Internal*	10 “	1	1	..	..
Internal*	12 “	1	..	1	..
Inunctions	2 months	2	2	..	..
“	5 “	1	..	1	..
“	6 “	2	2	..	..
“	1 year	1	1	..	..
Injections	4 injections	1	1	..	..
“	6 “	1	1	..	..
“	14 “	1	1	..	..
“	16 “	2	..	2	..
“	25 “	2	..	2	..
Injections and Inunctions	15 months	1	..	1	..
“ “ “	7 “	1	..	1	..
“ “ “	8 “	1	1	..	..

* Interrupted treatment during this time.

The consideration of this table makes evident the fact that a large number of patients upon internal treatment with mercury show a positive reaction after months or even years of such treatment, and that even treatment for a long period by injections or inunctions does not prevent the occurrence of a positive reaction in many instances.

The use of the test as a control of treatment has demonstrated the much greater specific value of arsenobenzol than of mercury. In many instances, the complement fixation reaction has been found positive after months or even years of mercurial treatment, but has quickly disappeared after the administration of arsenobenzol. I have selected from our data the most interesting cases of this kind and have included them in Table 6.

TABLE 6.

SHOWING THE EFFECT OF TREATMENT WITH ARSENOBENZOL UPON THE COMPLEMENT FIXATION TEST IN CASES SHOWING A POSITIVE REACTION AFTER MERCURIAL TREATMENT.

No.	Method of Mercurial Treatment	Length of Mercurial Treatment	Reaction	Dose of Arsenobenzol	Time of Disappearance of Reaction after Arsenobenzol
1.....	Internal	7 months	+	0.5 gm. intrav.	6 weeks
2.....	"	7 "	+	0.6 gm. intramus.	3 "
3.....	Injections and Internal	7 "	+	0.6 " "	9 "
4.....	Internal	7 "	++	0.6 " "	5 "
5.....	"	8 "	+	0.2 gm. intrav. 0.6 gm. intramus.	6 "
6.....	"	9 "	+	0.6 gm. intrav. 0.6 gm. intramus.	3 "
7.....	"	9 "	++	0.6 " "	6 "
8.....	"	1 year	++	0.6 " "	5 "
9.....	Inunctions and Injections	1 "	+	0.6 " "	8 "
10.....	Internal	1 "	++	0.6 " "	2 "
11.....	Injections and Internal	1 "	++	0.5 gm. intramus. 0.5 gm. intrav.	5 "
12.....	Internal	1 "	++	0.5 gm. intramus.	3 "
13.....	"	1 "	++	0.6 " "	5 "
14.....	"	1 "	++	0.6 " "	3 "
15.....	"	13 months	+	0.6 " "	7 "
16.....	"	18 "	++	0.6 " "	2 "
17.....	"	2 years	++	0.6 " "	8 "
18.....	Internal and Injections	2 "	+	0.6 " "	2 "
19.....	Internal	3 "	+	0.6 " "	3 "
20.....	Internal and 25 Injections	3 "	++	0.5 " "	7 "

It is evident from this table that arsenobenzol causes the complement fixation reaction to disappear in cases which have retained it under mercurial treatment for long periods of time, and when one considers that these cases were all resistant clinically to mercurial treatment the prompt disappearance of the reaction under arsenobenzol is remarkable.

Perhaps the best illustration of the ease with which one can use the test as a control of treatment is shown in experiments upon infected rabbits. Through the kindness of Captain Nichols, of the Army Medical Corps, I have been able to test the serum of rabbits infected with both lues and yaws, both before and after treatment with arsenobenzol. Table 7 shows the results obtained before and after treatment in a rabbit infected with lues, and Table 8 the results in a rabbit infected with yaws.

TABLE 7.

RESULTS OF COMPLEMENT FIXATION TEST BEFORE AND AFTER TREATMENT WITH ARSENOBENZOL IN RABBIT NO. 39, INFECTED WITH LUES.

AMOUNT OF SERUM	DATE OF TESTS								REMARKS
	Jan. 23	27	30	Feb. 6	8	10	13	18	
0.05 c.c.	−	+	+	++	−	+	o	−	Spirochaetes found in testicular lesion Jan. 19, 1911
0.10 c.c.	−	++	++	++	+−	++	−	−	
0.15 c.c.	o	++	++	++	+−	++	o	−	0.02 gm. arsenobenzol per kilo administered intravenously Feb. 6, 1911
0.20 c.c.	++	o	++	++	+−	++	−	−	

o = no test.

TABLE 8.

RESULTS OF COMPLEMENT FIXATION TESTS BEFORE AND AFTER TREATMENT WITH ARSENOBENZOL IN RABBIT NO. 44, INFECTED WITH YAWS.

AMOUNT OF SERUM	DATE OF TESTS								REMARKS
	Jan. 23	30	Feb. 2	6	8	10	13	17	
0.05 c.c.	−	−	++	++	−	−	−	−	Spirochaetes found in lesion Jan. 21, 1911
0.10 c.c.	−	+−	++	++	−	+−	−	−	0.02 gm. arsenobenzol per kilo administered intravenously Feb. 6, 1911
0.15 c.c.	−	+	++	++	+−	+	−	−	
0.20 c.c.	−	++	++	++	+−	++	−	−	

These tables illustrate how accurately one may follow the effect of treatment upon the complement fixation reaction, and in scores of our human cases we have been able to trace, week by week, the gradual disappearance of the reaction.

A negative reaction following treatment, unless it be repeatedly so, cannot be regarded as an indication that the infection is cured, for to be of value the test should be repeated at intervals of a month or two, for at least a year. If after thorough treatment the test remains negative for that length of time, I believe that it is justifiable to conclude that the infection is cured, provided the patient originally gave a positive reaction. *The presence of a reaction indicates active infection even though it may not be manifested by symptoms, and is always an indication that further treatment is necessary.* A negative result in the early period of the disease is of little value, but becomes more and more so the later the stage of the disease, the longer the duration of treatment, and the longer the reaction has remained negative. Many cases become negative after a short course of mercurial treatment, but practically all of these will give a positive reaction if the treatment be omitted for from three weeks to one month. It cannot be too strongly emphasized that a single negative reaction after treatment is of no value as an indication of cure except in patients who have been very thoroughly treated, and who have been free from symptoms for a year or more, and even in such cases it is best to have the test repeated at the end of another six months or a year.

Factors influencing the result of the complement fixation test.—I have already spoken of the influence of certain other diseases and of previous specific treatment upon the result of the complement fixation test, and desire, in conclusion, to call attention to certain other phenomena which have been observed in our routine application of the test and which are of both scientific and practical interest.

The relation of certain bacteria to the results of the test.—In recent experiments I have demonstrated[1] that certain strains of staphylococci and streptococci, when growing in normal serum, under favorable conditions, are capable of producing certain substances in the serum which cause a positive result with the complement fixation test for lues. In order that this may occur the bacteria must develop in the serum at incubator temperature for at least 24 hours or at room temperature for from five to seven days. In addition, every strain of a certain bacterial species will not produce this result as was shown by the fact that while a double-plus reaction occurred in normal serum infected with a stock *Staphylococcus aureus* and with a *Staphylococcus aureus* isolated from a normal serum giving a positive reaction, negative results were obtained with another *Staphylococcus aureus* isolated from a serum giving a negative reaction.

These non-specific reactions do not occur in normal sera which are sterile, even though the sera be kept at room temperature for as long a period as one month. Such reactions are in all probability very rare in practice, but the fact that they can occur as a result of bacterial activity is of enough importance to justify the use of aseptic methods in the collection of blood for the Wassermann test. In two sera which gave a negative reaction on the first test, infection with a streptococcus caused the reaction to become positive a week later, and in the only instance we have observed in which a presumably normal serum, on the first test, gave a positive reaction, bacterial contamination was probably the cause.

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[1] *Jour. Exp. Med.*, 1911, 13, p. 521.

The effect of the ingestion of alcohol upon the results of the test.—Working in conjunction with Captain Nichols, of the Army Medical Corps, it has been demonstrated[1] that the ingestion of considerable amounts of alcohol will render a positive serum negative if it be tested within 24 hours after alcohol has been administered, and in some cases the serum will give a negative reaction for three days after the administration of this drug. We tested nine individuals, all of whom gave a double-plus complement fixation test, administering quantities of alcohol varying from 90 c.c. to 120 c.c. in the form of beer and whiskey, and found that in every case the positive reaction disappeared within 24 hours after the administration of the drug, and that in one case it did not become positive again until the end of three days, while in the others the serum again became positive within 36 hours.

These observations have demonstrated that no dependence can be placed on a negative Wassermann reaction in individuals who have, within 24 hours of the collection of the blood, ingested a considerable amount of alcohol, while in some instances the drug may render the reaction negative for as long a period as three days. All of our tests were made upon individuals giving absolute inhibition of hemolysis and it is probable that the drug will be found to have a still more decisive effect upon weaker reactions, even though given in much smaller quantities. Experiments are now under way to determine the smallest amount of alcohol which will render the test negative and the effect of the drug upon cases giving a plus or a plus-minus reaction. In view of the effect of alcohol upon the complement fixation test a careful inquiry should always be made regarding the recent use of this drug before collecting blood for the complement fixation test.

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[1] *Jour. Am. M. Ass.*, 1911, 52, p. 474.

In the military service the complement fixation test has proved of the greatest value in the diagnosis of obscure and latent infections; in preventing the enlistment of luetic individuals; and in controlling treatment both with mercurials and arsenobenzol. The test is being used as a routine measure in the laboratories of the Medical Department of the army both in this country and the Philippine Islands, and continued use has only increased our confidence in its value as a diagnostic measure.